BIOLOGY AND TRANSLATIONAL RESEARCH. ORAL POSTER PRESENTATIONS
12th June- 15th October 2020

S165 Oral Presentation. IDENTIFICATION OF A DRUG-ABLE DOPAMINE RECEPTOR MEDIATED PATHWAY THAT IS CRITICAL FOR CML STEM CELL SURVIVAL

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EHA Library. Ritchie J. 06/13/20; 294985; S165
Session title: Mechanisms and strategies to address TKI resistance and stem cell persistance in CML

Background
One of the most significant obstacles in developing a cure for chronic myeloid leukaemia (CML) is the existence of TKI-insensitive leukaemic stem cells (LSCs) that can drive relapse, TKI resistance and disease progression. Haematopoietic stem cells (HSCs) are found within the bone marrow microenvironment where signalling from the sympathetic nervous system is known to have important roles in their survival and maintenance. Our previous transcriptomics analysis pointed to neurotransmitter pathways as being active in LSCs, leading us to hypothesise that these pathways may also have critical roles in LSCs.

Aims
To identify drug-able components of neurotransmitter signalling that are critical for the survival of CML LSCs.

Methods
Phenotypic medium-throughput primary compound screens were performed in CML cell lines with 658 neurotransmitter-modulating compounds, whilst secondary screens confirmed selectivity towards primary CML CD34+ cells versus non-CML CD34+ cells. Our lead compounds (see below) were taken forward into phenotypic assays of CML and non-CML CD34+ cells to further examine its effects in vitro on cell expansion, apoptosis, and CFC (colony forming cell) and LTC-IC (long term-colony initiating cell) assays. The efficacy of compounds on CML cell survival was also assessed in vivo using patient-derived xenograft (PDX) and transgenic murine models of CML. To investigate the mechanism of action of lead compounds, genetic (shRNAs) and pharmacological perturbations, in combination with phenotypic assays (as above), flow cytometry and western blotting was used.

Results

We identified compounds targeting the dopamine signalling pathway as the most over-represented set in our screens with efficacy at selectively affecting CML cell survival ($p = 9.5e-8$). Among these, the D2-like dopamine receptor antagonist fluphenazine was identified as having the greatest therapeutic potential. While fluphenazine had very little effect on the bulk CD34+ leukaemic cells, the effects on the more primitive CD34+38- cells were strikingly much more pronounced and led to significant reductions in cell counts ($p < 0.001$), CFC outputs ($p<0.001$) and increased apoptosis ($p<0.05$) when used as a single agent or in combination with nilotinib. LTC-IC assays revealed that fluphenazine selectively eradicates LSCs, reducing LTC-IC output to 5% compared to vehicle ($p<0.001$) whilst having no observable effects on LTC-IC output of HSCs. Fluphenazine when combined with nilotinib is highly effective at eradicating LSCs compared to nilotinib alone in vivo in both PDX and transgenic mouse models of CML ($p<0.05$-0.01).

LSCs express two of the D2-like dopamine receptors (DRD3, and DRD4) at significantly higher levels compared to HSCs ($p< 0.05$-0.001) suggesting that fluphenazine's mechanism of action in CML cells is through these receptors. Indeed, genetic knockdowns -/+ TKI have shown that DRD3 and DRD4 are important for CML cell survival ($p<0.01$). Further mechanistic studies in CML cell lines and in primary CD34+ cells indicate that fluphenazine -/+ TKI disrupts signalling through a pathway involving PRKCH, ERK1/2, c-MYC and FOXO3a. On-going global RNA-seq studies will provide us with additional mechanistic insights on downstream targets of c-MYC and FOXO3a that may also play roles in the mechanism of action of fluphenazine.

Conclusion

We have demonstrated that the receptors DRD3 and DRD4 play key roles in an important LSCs survival pathway. Targeting this pathway using D2-class antagonists such as fluphenazine represents a novel way of eradicating LSCs.

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S166 Oral Presentation. MODEL-BASED INFERENCE AND CLASSIFICATION OF IMMUNOLOGICAL CONTROL MECHANISMS FROM TKI CESSATION AND DOSE REDUCTION IN CML PATIENTS

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EHA Library. Glauche I. 06/13/20; 294986; S166
Session title: Mechanisms and strategies to address TKI resistance and stem cell persistance in CML

Background

Recent clinical findings in chronic myeloid leukemia (CML) patients suggest that the risk of molecular recurrence after stopping tyrosine kinase inhibitor (TKI) treatment substantially depends on an individual's leukemia-specific immune response. However, it is still not possible to prospectively identify patients that will remain in treatment-free remission (TFR).

Aims

To adapt a patient specific, mathematical model of CML-immune interactions and to apply and verify it for alternative treatment scenarios.

Methods

We suggest a mathematical model for CML, which explicitly includes an anti-leukemic immunological effect and apply it to time course data of 21 CML patients for whom BCR-ABL1/ABL1 measurements have been quantified before and after TKI cessation. Fitting the model simulations to data, we identify patient-specific parameters that allow patient classification as well as model predictions for alternative treatment scenarios, i.e. intermediate dose reduction before treatment cessation.

Results

Implementing immunological control in our mathematical CML model was conceptually necessary to explain TFR as observed in about half of the patients. Fitting the model simulations to data, we identify patient-specific parameters and classify patients into three different groups according to their predicted immune system configuration ('immunological landscapes"). While one class of patients required complete CML eradication to achieve TFR, other patients were able to control residual leukemia levels after treatment cessation. Among them were a third class of patients, that maintained TFR only if an optimal balance between leukemia abundance and immunological activation was achieved before treatment cessation. We further apply the model to study changes in the BCR-ABL1 dynamics resulting from intermediate TKI dose reduction. Our results indicate that the linear slope of the BCR-ABL1 ratios correlates with the risk of recurrence after TKI stop (OR: 1.21, 95% CI: 1.07–

1.51) and conveys information about the patient-specific immune system. Our results are in qualitative and quantitative agreement with a recent reanalysis of clinical data from the DESTINY trial (NCT01804985), for which we could demonstrate that the patient-individual slope of BCR-ABL1/ABL1 ratios monitored during TKI dose reduction strongly correlates with the risk of individual recurrence after TKI stop (OR: 1.28; 95% CI: 1.17-1.42).

Conclusion

This inference of individual immunological configurations based on treatment alterations underlines the importance of understanding immunological control mechanisms and acts as a showcase for other cancer types in which the endogenous immune system supports maintenance therapy, long-term disease control or even cure.

References:

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Gottschalk A, Glauche I, Cicconi S, Clark RE, Roeder I. Molecular dynamics during reduction of TKI dose reliably identify molecular recurrence after treatment cessation in CML. Blood. 2020 Jan 14. doi: 10.1182/blood.2019003395. [Epub ahead of print]

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S167 Oral Presentation. MECHANISTIC AND THERAPEUTIC INSIGHTS INTO PRC2 REPROGRAMMING IN BLAST PHASE CML

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EHA Library. Mitchell R. 06/13/20; 294987; S167
Session title: Mechanisms and strategies to address TKI resistance and stem cell persistance in CML

Background

Chronic myeloid leukaemia (CML) is a clonal haematopoietic stem cell disease driven by the constitutive expression of BCR-ABL1. Most CML patients present with chronic phase disease (CP-CML), however a minority present with, or progress to, blast phase disease (BP-CML) which has a median survival of 6-11 months. Whilst tyrosine kinase inhibitors (TKIs) have transformed clinical outcomes for most CP-CML patients, TKIs have only transient efficacy in BP-CML, and other treatment options are limited. Thus, the development of targeted therapeutic strategies for BP-CML remains an area of unmet clinical need in the management of CML. Previously, our group has shown that Polycomb Repressive Complex 2 (PRC2) is dysregulated in CP-CML and that extensive reprogramming of H3K27me3 targets sensitizes leukaemic stem cells (LSCs) to EZH2 inhibitors (EZH2i). Here, we extend our analysis of this epigenetic vulnerability to BP-CML, where our data supports that inhibition of PRC2 activity is a novel therapeutic strategy.

Aims

To determine whether PRC2 re-programming in BP-CML results in an epigenetic vulnerability that can be targeted therapeutically.

Methods

mRNA levels of PRC2 components and the global epigenetic landscape were assessed in diagnostic myeloid BP-CML $CD34^+$ cells. Dysregulated PRC2 components were targeted in vitro using lentiviral-mediated knockdowns in two myeloid BP-CML cell lines and, in parallel, the efficacy of an EZH2i (EPZ011989) alone and in combination with nilotinib (NIL) in BP-CML $CD34^+$ cells was assessed in vitro and in vivo using murine models. The phenotypic and molecular effects of these perturbations was examined by monitoring cell counts, colony-forming cell (CFC) outputs, the levels of apoptosis, and by RNA-seq.

Results

Several PRC2 components are transcriptionally dysregulated in BP-CML $CD34^+$ cells when compared to normal $CD34^+$ haemopoietic samples (n=7; $p<0.05$), including PHF1, MTF2 and RBBP4, which are not known to be dysregulated in CP-CML. Similarly, the global H3K27me3 landscape in BP-CML

shows unique patterns compared to CP. Knockdown of several PRC2 components resulted in substantial reduction in cell expansion and increased apoptosis when compared to scrambled (scr) controls. Taken together this data suggests the PRC2 membership and occupancy has unique features in BP which we are further exploring by RNA-seq to elucidate target preferences for PRC2 components.

Primary $CD34^+$ cells from BP-CML patients showed significantly reduced cell expansion ($n=3$; $p<0.0001$) and a trend toward increased apoptosis when treated with the combination of EPZ011989 and NIL, relative to vehicle. The combination of EPZ011989 with NIL showed a 96% reduction in CFC output following combination treatment of BP-CML $CD34^+$ cells compared to vehicle ($n=3$; $p<0.0001$). Our previous work has shown minimal effects of EZH2i on cell survival, apoptosis and CFC output in normal $CD34^+$ cells in vitro. In long-term bone marrow patient-derived xenografts of BP-CML samples, the combination of EPZ011989 and NIL showed a significant reduction in the engrafted leukaemic stem and progenitor cells compared to vehicle ($n=2$ BP-CML samples; $p<0.003$) which exceeded the effects of NIL treatment alone. The molecular responses for this drug combination in CP and BP $CD34^+$ cells is currently being examined using RNA-seq.

Conclusion

Overall, this study provides compelling evidence that PRC2 is a tractable target in both CP- and BP-CML and a rationale for evaluating these in ongoing and future CML clinical trials.

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S168 Oral Presentation. MECHANISTIC INSIGHTS INTO THE INHIBITION OF T REGULATORY CELLS BY DASATINIB IN CML PATIENTS WITH CLONAL LYMPHOCYTOSIS

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EHA Library. Harrington P. 06/13/20; 294988; S168
Session title: Mechanisms and strategies to address TKI resistance and stem cell persistance in CML

Background

Dasatinib is a potent inhibitor of Lck, which plays a pivotal role in signalling from the T cell receptor (TCR), with immediate downstream targets including ZAP70 and LAT, which are also implicated in Treg development. STAT5, the downstream target of IL-2, also plays a critical role in Treg differentiation and maintenance of FOXP3 expression through its binding of the promoter region of the FOXP3 gene. A subset of patients on dasatinib develop a clonal large granular lymphocytosis (LGL) which is associated with immune-mediated toxicity and improved outcome.

Aims

We hypothesised that a reduction in Treg frequency and function would correlate with the expansion of a clonal LGL population in certain patients taking dasatinib.

Methods

We performed phosphoflow cytometry in Tregs and Teffectors to assess the effect of dasatinib on signalling from the TCR. Cells were activated with the phosphatase inhibitor H2O2 for 15 minutes and were analysed for phosphorylation of ZAP70, LAT and STAT5. A gating strategy of CD4+/CD25+/FOXP3+/CD127lo cells was used for identification of Tregs, with FOXP3hi/CD45RA-ve cells denoting effector Tregs (eTregs). Intracellular flow cytometry was also performed on Teffectors after stimulation with OKT3, assessing the impact of dasatinib on cytokine expression, including TNFα, IFNγ, IL-2, IL-4 and IL-10.

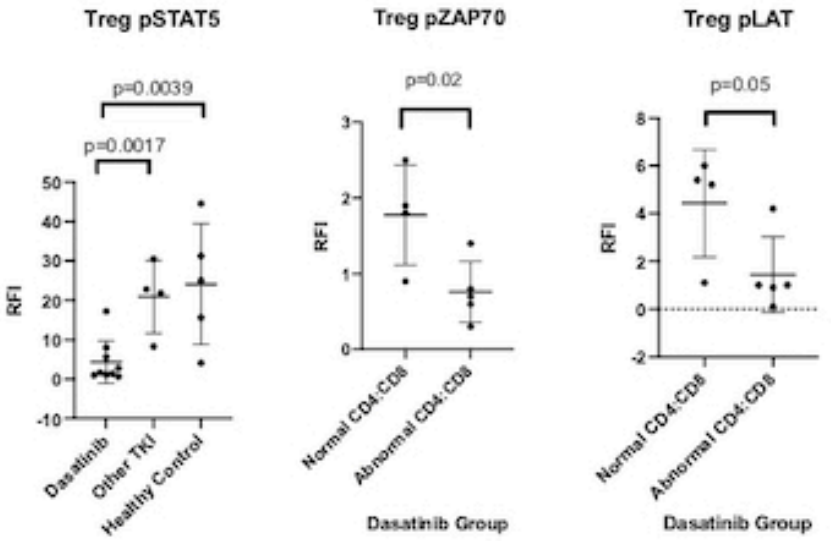
Results

15 patients with CML (dasatinib n=11, imatinib/nilotinib n=4) and 5 healthy controls were recruited. Patients on dasatinib had lower Tregs compared with the non dasatinib group (mean % of CD3+ cells 1.3 vs 2, mean % of CD4+ cells 2.6 vs 4.3, p=0.01 and 0.007). Dasatinib treated patients also had a lower % of eTregs 11.5 vs 22.1 (p=0.009)

Patients on dasatinib had significantly reduced phosphorylation of ZAP70, LAT and STAT5 compared with the non-dasatinib group, in CD4+ cells, CD8+ cells and Tregs, following stimulation (pSTAT5 mean increase in MFI of 4.1 vs 21.7 in CD4+ cells, 6.1 vs 28.2 in CD8+ cells and 4.4 vs 22.6 in Tregs (p=0.0001, 0.0001, and 0.001).

Five patients on dasatinib had reversal of CD4:CD8 ratio and lymphocytosis, in keeping with clonal LGL populations (with TCR clonality confirmed by PCR). These patients had lower Tregs as a % of CD3+ cells when compared with other patients on dasatinib with a mean of 0.9 vs 1.8 (p=0.035). Importantly, a lower increase in MFI within isolated Tregs following stimulation was seen in this group, when compared with patients on dasatinib with normal CD4:CD8 (pZAP70 1.8 vs 0.8, p=0.024; pLAT 4.4 vs 1.4, p=0.05; pSTAT5 7.4 vs 2, p=0.15).

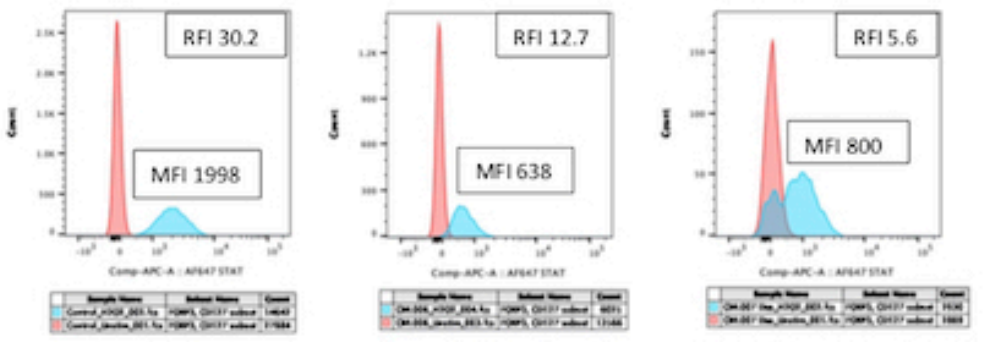
Mean absolute increase in IL-2 expression was lower in patients on dasatinib (0.9 vs 7 in CD4+ cells and 0.3 vs 3 in CD8+ cells, p=0.001 and 0.014) and those with reversal of CD4:CD8 also had reduced increase in IL-2 expression compared with other patients on dasatinib.



RFI – Relative fluorescence intensity (MFI H2O2/MFI Unstimulated)

pSTAT5 within Tregs:

Red peak – Unstimulated, Blue peak – H2O2 stimulation



Control | dasatinib, no lymphocytosis | dasatinib, CD8+ lymphocytosis

Conclusion

Dasatinib potently inhibits signalling from the TCR in Teffectors but also in Tregs, as indicated by inhibition of phosphorylation of ZAP70 and LAT, as well as STAT5, which is essential for transcription of FOXP3. Dasatinib treated patients have a reduction in proinflammatory cytokine expression within Teffectors, with the most significant inhibitory effect seen against IL-2. Tregs have abundant expression of the IL-2 receptor on the cell surface and binding leads to STAT5 signalling.

A subset of patients on dasatinib with clonal LGL populations have further reduction in frequency and function of Tregs as assessed by signalling from the TCR and IL-2R. These findings may explain the mechanism of lymphocytosis in this group and could be used to predict improved outcomes with dasatinib.

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S169 Oral Presentation. PERIPHERAL BLOOD CD26+ LEUKEMIA STEM CELLS AND TKI DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA PATIENTS: INTERIM ANALYSIS OF PROSPECTIVE FLOWER-TFR STUDY

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EHA Library. Sicuranza A. 06/13/20; 294989; S169
Session title: Mechanisms and strategies to address TKI resistance and stem cell persistance in CML

Background

Given the evidence that deepness and duration of molecular response are necessary but not sufficient requisites for a successful treatment free remission (TFR) additional biological criteria to possibly identify more and better CML patients suitable for an efficacious discontinuation are today focus of research in CML. Leukemia stem cells (LSCs) are supposed to be the reservoir of disease. We first showed in a cross-sectional study that residual circulating CD34+/CD38-/CD26+ CML-specific LSCs are still detectable in the majority of CML patients in sustained TFR (66%) with a median value of 0.015μ/L despite stable and deep molecular response.

Aims

In prospective FLOWER-TFR multicentered study (AIRC IG 20133) we monitored by flow-cytometry the number of circulating CD26+ LSCs in CML patients from the time of TKI discontinuation until molecular relapse, if any.

Methods

CML patients meeting the current molecular criteria for TKI withdrawal entered this multicenter study. At time of stopping TKI treatment (baseline) and at +1, +2, +3, +6, + 12 months after discontinuation and at any time of molecular relapse, CML patients were evaluated for number of peripheral blood CD34+/CD38-/CD26+ LSCs by centralized flow-cytometry analysis and for BCR-ABL transcript level by standard (IS) quantitative RT-PCR assay.

Results

Up to date, 71 consecutive CML patients were enrolled in the study. Patients characteristics and results are summarized in Table 1. After a median observation time of 11 months since TKI withdrawal (1-37 months), 20/71 (28%) patients lost their molecular response and restarted TKI treatment while 51/71 (72%) are still in TFR; however 12/51 (23%) patients have so far discontinued the treatment for ≤ 6 months. The median time to relapse after discontinuation was 4 months (range 2-7 months). At the time of discontinuation, residual CD26+LSCs, were detectable in 36/71 (51%) patients of the whole cohort; in 24/51 (47%) patients that sustained TFR and in 12/20 (60%) of those that

subsequently loss response (table 1). The median number of detected circulating CD26+LSCs was 0.0237µ/L (range 0.0077-0.1197) with minimal fluctuation at different time points. Even in those patients with no detectable CD26+ LSCs at time of discontinuation (35/71, 49%) we could document in most of them, the persistence of circulating residual CML LSCs at least one time. Kendall rank correlation coefficient, Mood test and bi-linear relation model of the whole cohort showed no correlation between BCR-ABL/ABLIS ratio and number of residual CD26+ LSCs either at baseline or at each time points after discontinuation, thus confirming our previous observations.

TOTAL PATIENTS		71
Median age at diagnosis		68 (19-71)
Sex	Male	39 (55%)
	Female	32 (45%)
Sokal skore	High	10/71 (14%)
	Intermediate	21/71 (29%)
	Low	36/71 (51%)
	n.a.	4/71 (6%)
Type of TKI	IMATINIB	43
	NILOTINIB	20
	DASATINIB	8
Median TKI treatment duration before discontinuation (months, range)		103 (38-232)
Median duration of treatment according to TKI (months, range)	IMATINIB	124 (38-232)
	NILOTINIB	92.5 (50-151)
	DASATINIB	65.5 (59-170)
Measurable circulating CD26+ LSCs at time of discontinuation	YES	36/71 (51%)
	NO	35/71 (49%)

	TOTAL (71)	TFR SUSTAINED (51)	TFR LOSS (20)
CD26LSC+ detectable BCR-ABL/ABL ratio detectable	10/71 (14%)	6/51 (12%)	4/20 (20%)
CD26LSC+ detectable BCR-ABL/ABL ratio undetectable	26/71 (37%)	18/51 (35%)	8/20 (40%)
CD26LSC+ undetectable BCR-ABL/ABL ratio undetectable	19/71 (27%)	15/51 (29%)	4/20 (20%)
CD26LSC+ undetectable BCR-ABL/ABL ratio detectable	16/71 (22%)	12/51 (24%)	4/20 (20%)

Conclusion

The interim analysis of this unique study confirms that CD26+ LSCs are detectable at time of TKI discontinuation and during TFR. Moreover, at least for the observation median time of the study (11 mos) the persistence of “fluctuating” values of residual CD26+ LSCs do not hamper the possibility to maintain a stable TFR. On the contrary, even patients discontinuing TKI with no detectable CD26+ and no detectable BCR-ABL undergo TFR loss (4/20, 20%). Our preliminary results suggest other factors then residual LSCs “burden” playing an active role in controlling disease recurrence. Additional studies evaluating CD26+ LSCs ability to modulate the immune system through a variable expression of immune response inhibitory molecules and through their interactions with effectors cells are ongoing.